Iranian Journal of Veterinary Science and Technology
Vol. 5, No. 2, 2013, 33-44

IJVST

Effects of different levels of *Aloe vera* L.extract on growth performance, hemato-immunological indices of *Cyprinus carpio* L.

Mojtaba Alishahi[1*], Esmail Abdy[2]

[1] *Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran*
[2] *PhD Student of Aquatic Animals Health, Veterinary Faculty of Tehran University, Tehran, Iran*

Received: November 13, 2012 Accepted: November 1, 2013

Abstract

This study was conducted to determine the effects of administration of different concentration of *Aloe vera* Crud Extract (ACE) as a food supplement on growth performance, immune response and disease resistance against *Aeromonas hydrophila (Chester)* infection in *Cyprinus carpio*. 360 juvenile *C.carpio* were randomly divided into four equal groups in triplicates. Groups 1 to 4 were fed by basal food supplemented with zero, 0.1, 0.5 and 1% ACE respectively, for 60 days. At the end of the experimental period growth performance indices including: SpecificGrowth Rate (SGR), Food Conversion Rate (FCR), Food Efficacy Rate (FER) and Percentage Weight-Gain (PWG) were evaluated. Blood samples were taken from each treatment and Haematological parameters including: Red Blood Cells (RBC) & White Blood Cell(WBC) count, differential count, Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC) as well as Immunological parameters including: lysozyme activity, serum bactericidal activity, alternative complement activity, respiratory burst activity, serum total protein and globulin were evaluated. Finally challenge with *Aeromonas hydrophila* was performed and commutative mortality was calculated.

Results showed significant improve in growth indices of groups 3 and 4 ($p<0.05$). PCV and MCV, as well as most of the immune parameters including, lysozyme activity, complement activity, respiratory burst activity and serum bactericidal activity were significantly ($p<0.05$) higher in groups 3 and 4. In addition the rate of challenge mortality was significantly lower in the 0.5 and 1% ACE treated fishes than other groups ($p<0.05$). In conclusion supplementation of food with 0.5 and 1% ACE has immunostimulating, growth stimulation effects, as well as resistance against bacterial infection in *C.carpio*.

Keywords: *Aloe vera* extract, *Cyprinus carpio*, *Aeromonas hydrophila*, immunostimulant, hemato-immunological parameters

* Corresponding author: Mojtaba Alishahi
Email: alishahim@scu.ac.ir
P.O.: 61335-145

Introduction

As fishes are placed in lower levels of evolutionary system, non-specific immunity has more important role against pathogens in fishes, so the use of immunostimulatory agents seems to have a good result in fish immune response (Zapata *et al.*, 2006; Swain *et al.*, 2007). Herbal immunostimulants have numerous potential benefits in comparison to vaccines and drugs like antibiotics. They are not expensive and are available almost all around the world; in contrast vaccines are generally expensive and are not available for all kinds of disease (Raa *et al.*, 1992 and Rojhan, 2007). Nowadays abuse of antimicrobial drugs and disinfectants in aquaculture has obviously led to the evolution of resistant strains of bacteria (Kim *et al.*, 1999), but fortunately this is not the case with herbal immunostimulants (Raa, 1996). Therefore, using immunostimulants seems to be an attractive alternative way of controlling fish diseases and recently the use of immunostimulants in fish farms has been becoming popular for enhancing the activity of nonspecific defense mechanisms and conferring protection against diseases (Raa, 1996).

Aloe vera L. Burm.f or *Aloe barbadensis* Miller is a tropical member of the lily family (*Liliaceae*) which is characterized by lance-shaped leaves. *Aloe vera* is recognized for its widespread use and reported healing powers (Krishnan, 2006), alleviating pain and treating a variety of ailments (Shelton, 1991).

Acemannan (a kind of polysaccharide), is the main part and functional component of *Aloe vera*'s gel (Lee *et al.*, 2001). The refined polysaccharide has been shown to act as an immunostimulant, displaying adjuvant activity and enhancing the release of different kinds of cytokines (Peng *et al.*, 1991) as well as stimulating hematopoiesis (Egger *et al.*, 1996). Acemannan have been reported to have anti-microbial properties, including anti-bacterial, anti-fungal, anti-viral, and anti-parasitic properties (Boudreau and Beland, 2006).

Although the immunomodulatory potentials of *Aloe vera* in mammals particularly in human have been well confirmed (Tan and Vanitha, 2004), few works were done on effects of *Aloe vera* on fish (Kim *et al.*, 1999 and Alishahi *et al.*, 2010).

Common carp is one of the most important fishes in the world and Iran aquaculture. Recently various mass mortalities were reported in carp culture of Iran (Sattari, 2008) and use of immunostimulants is a suggestible alternative to antibiotics in this species.

In this study the growth performance indices and hematoimmunological parameters of *Cyprinus carpio* were investigated fallowing feeding different doses of ACE.

Materials and methods

Fishes

360 juvenile common carps, weighing an average of 45±3 gr were purchased from a cyprinid fish farm in Ahvaz, Khuzestan province, south Iran. Water quality parameters including water temperature, dissolved oxygen, pH, NH_3 and NO_2 were, 26±1°C, 9±1 mg l^{-1}, 7.7±0.33, <0.01 mg l^{-1} and <0.1 mg l^{-1}, respectively. Fish were acclimatized for two weeks prior to the experiment and during experiment period, physicochemical parameters of water was checked and maintained in standard range by using of automatic aquarium heaters, bio-filters and frequent water changes.

Aloe Extract

Crude extracts of *Aloe vera* were prepared by method that has been described previously by Alishahi *et al.* (Alishahi *et al.*, 2010). Briefly the process was removing the thick green outer layer of Aloe leavs, extracting the inner gel and purifying it by squeezing, homogenating and filtering.

Grouping

Fishes were randomly divided into four groups (in triplicate) of 90 fishes and each group divided to triplicates of 30. Each of 12 treatments placed into 150 L aquarium equipped with thermostat controlled heaters, aeration, external biofilters and automatic feeders.

Experimental food preparation

The commercial common carp food (Beiza

Co, Iran) were used as a basal diet. For better homogenation of ACE with food, initially granulated food converted to paste by adding distilled water in it, then 0.1, 0.5 and 1% (w/w) ACE added to food and homogenized with electric mixture. Finally food pelleted by means of special meat grinder. Control food prepared in the same way without any supplementation of ACE. Prepared experimental foods were packed in nylon bags, labeled and stored at 4 ºC until use.

Growth performance

The Percentage Weight-Gain (PWG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Food Efficiency Ratio (FER) were calculated according to the following equations (Azza, 2009):

PWG (g/fish) = [Average final weight - Average initial weight] / initial weight

SGR (%/day) = [final body weight - ln initial body weight] × 100 / experimental period (day).

FCR= Food intake / weight gain.

FER = Body weight gain / Food intake.

(All of the fish weights in top equations were calculated in gram unit).

Blood and serum sampling

At the end of the feeding experiment, fish anaesthetized by immersing in water containing 70 ppm concentration of MS-222. Blood-samples were collected from the caudal vein, by using needles impregnated with heparin for the evaluation of hematocrit, white blood cell count (WBC) and WBC differential count (Thrall, 2004). For serum separation, another blood-sample was withdrawn from the caudal vein; into blood Eppendorf tubes without anticoagulant in syringe. The Eppendorf tubes, containing the blood samples were centrifuged at 2500 g for 15 min and supernatant serum was collected. The serum was stored at -20 Cº in Eppendorf tubes until use for immunological studies.

Lysozyme activity

The lysozyme activity was measured using photoelectric colorimeter equipped with attachment for turbidity measurement. A series of dilution was prepared by diluting the standard lysozyme from hen egg-white (Sigma) and mixed with *Micrococcus lysodeikticus* (Schroeter) (Sigma) suspension for establishing the calibration curve. Ten µl of standard solution or serum were added to 200 µl of micrococcus suspension (35 mg of Micrococcus dry powder/95 ml of 1/15 M phosphate buffer + 5.0 ml of 1M NaCl solution). The changes in the extinction were measured at 546 nm by measuring the extinction immediately after adding the solution which contained the lysozyme (start of the reaction) and after 20 min incubation of the preparation under investigation at 40 Cº (end of the reaction). The lysozyme content is determined on the basis of the calibration curve and the extinction measured (Thrall, 2004).

Serum bactericidal activity (SBA)

Bactericidal activity was studied following procedure by (Azza, 2009) with slight modification. Sera samples were diluted three times with 0.1% gelatin-veronal buffer (GVBC2) (v/v), (pH 7.5, containing 0.5 mM ml^{-1} Mg^{2+} and 0.15 mM ml^{-1} Ca^{2+}). *A. hydrophila* (live, washed cells) suspended in the same buffer at concentration of 10^5 CFU ml^{-1}. The diluted sera and bacteria were mixed at 1:1 v/v, incubated for 90 min at 25 ºC with shaker. Control group containing bacterial suspension was also included. The number of viable bacteria was then determined by counting the colonies after culturing on Tripticase Soy Agar (TSA) plates for 24 h at room temperature 25 ºC.

Respiratory burst assay

The respiratory burst activity was measured by the reduction of Nitro Blue Tetrazolium (NBT) by intracellular superoxide radicals (Anderson and Siwicki, 1994). Briefly, 100 ml of heparinised blood from fish of each group was mixed with 100 ml of 0.2% NBT (Merk, Germany) solution for 30 min at 25 ºC. After incubation, 50 ml of the above mixture was added to 1 ml of N,Ndiethylmethyl formamide (Sigma, USA) and then centrifuged at 3000 g for 5 min. The optical density of the supernatant was measured at 540 nm and

compared among treatments.

Alternative complement activity

The activity of the alternative complement pathway was assayed using Sheep Red Blood Cells (SRBC) as targets (Ortuno *et al*., 1998). SRBC were washed in phenol red free hank's balanced salt solution (HBSS) containing Mg2+ and EGTA and resuspended at 3% in HBSS. Aliquots (500 µl) of test serum as complement source, diluted in HBSS, was added to 500 µl of SRBC to give final concentrations of 10, 5, 2.5, 1.25, 0.625, 0.313, 0.1565 and 0.078%. After incubation for 1 h at 22 ºC, the samples were centrifuged at 800 g for 5 min at 4 ºC to remove non-lysed erythrocytes. The relative haemoglobin content of the supernatants was assessed by measuring their optical density at 540 nm in a spectrophotometer. The values of maximum (100%) and minimum (spontaneous) haemolysis were obtained by adding 500 µl of distilled water or HBSS to 500 µl samples of SRBC, respectively. The degree of haemolysis (Y) was estimated and the lysis curve for each specimen was obtained by plotting Y/1 - Y against the volume of serum added (ml) on a log-log scaled graph. The volume of serum producing 50% haemolysis (ACH50) was determined and the number of ACH50 units ml-1 was obtained for each experimental group.

Total serum protein and globulin

The total serum protein level was estimated by the method of Bradford (Bradford, 1976) using the standard protein estimation kit (Zist Shimi Co, Iran). For globulin estimation, 50 ml saturated ammonium sulphate solution was added drop wise to 50 ml serum followed by vortexing. Centrifugation was done at 10,000 g for 5 min. Then 20 ml of this sample dissolved with 80 ml carbonate-bicarbonate buffer (pH 9.3) and the protein content was estimated through the method of Bradford using the standard protein estimation kit (Zist Shimi Co, Iran).

Hematological parameters

Blood samples immediately analyzed for the estimation of numbers of erythrocytes and, Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC). Number of erythrocytes was determined by the hemocytometer method (Ellis, 1990); haematocrit was determined by the microhematocrit method (Fox, 1997) and hemoglobin measurement was determined by the cianometa-haemoglobin method (Goldenfarb, 1971). MCV, MCH and MCHC were calculated by using the formulas as follow (Hu, 2005) :

MCV (μm^3 $cell^{-1}$) = (Packed cell volume as percentage/RBC in millions cell mm^3)$\times$10

MCH (pg $cell^{-1}$) = (Hb in g 100 ml^{-1}/ RBC in millions cell mm^3) $\times$10

MCHC (g 100 ml^{-1} Hct) = (Hb in g100 mL^{-1}/ packed cell volume as percentage) $\times$100

White blood cell count (WBC) and Differential count was made from 6 animals of each group in a Neubauer counting chamber as described by Schaperclaus (Schaperclaus *et al.*, 1991). For Differential count of leukocytes whole blood on glass microscope slides, dried in air, and stained with May-Grunwald/Giemsa. leucogram was assessed for each fish under an oil immersion lens. One hundred white blood cells from each smear were assessed and the percentage of different types of leucocytes was calculated following the method of Schaperclaus (Schaperclaus *et al.*, 1991).

Challenge with Aeromonas hydrophila

After the 60 days of the feeding experiment, 10 fish from each aquarium were challenged intraperitoneally with 0.2 ml of 2$\times LD_{50}$ concentration of live *A. hydrophila*. The challenged fish were kept under observation for 10 days. Cause of death was ascertained by re-isolating the *A.hydrophila* from kidney and liver of dead fish. The mortalities were recorded daily for 14 days and mortality percentages were compared among the groups.

Statistical analysis

Data are presented as Mean±SD of the number of fish per group. Growth indices,

hematological and immune parameters were analyzed using the one way ANOVA followed by Duncan's multiple range test to compare the difference in values between Aloe treated and control fish using the statistical package (SPSS).

Results

Growth performance

Results of growth parameters are shown in table 1. The use of 0.5% and 1% of ACE (Groups 3&4) concentrations had a significant effect on enhancement of growth parameter including Growth Rate, SGR and FCR (p<0.05). No significant difference was seen between 0.1% group and control (p>0.05). Mortality was same for all groups.

Lysozyme activity

The serum lysozyme activity significantly increased with ACE 0.5% and 1% (Fig.1). Group 2 did not show any difference in lysozyme activity with control treatment.

Serum bactericidal activity (SBA)

As shown in table 1, the use of 0.5% and 1% of ACE, significantly decreased the count of bacterial colonies in comparison to 0.1% ACE and control group (p< 0.05).

Respiratory burst assay (obtained from NBT reduction)

The production of superoxide, examined by NBT reduction, was significantly higher in blood leukocytes of fishes in group 3 than leukocytes of the groups 1 and 2 (p< 0.05). This parameter was also higher in group 4 than groups 1 and 2, but the difference was not significant (p>0.05).

Alternative complement activity

According to results that are shown in table 1, groups 3 and 4 had more compelent activity than groups 2 and 1 (p<0.05).

Total serum protein and globulin

Highest levels of total serum protein and globulin were seen in groups 3 and 4 which have a significant difference with groups 1 and 2 (table 1).

Hematological parameters

The results of hematological parameters of treatments are shown in table 1.

In PCV and MCV measurement, groups 0/5% and 1% ACE show significantly higher levels of PCV and MCV than 0/1% ACE and control.

In Hb measurement and RBC count, 1% ACE was the only group which had a significant difference with control and other ACE concentrations.

No significant differences were seen between four groups in other hematological parameters (i.e MCH, MCHC, Heterophil, Lymphocyte, Monocyte and Eosinophil count).

Discussion

Immuno stimulating effects of many herbal medicines in various fish species is proven. Enhancement of immune responses of *Cyprinus carpio* (Jian and Wu, 2004), *Oncorhynchus mykiss* (Dügenci *et al.*, 2003), *Carassius auratus gibelio* (Chen *et al.*, 2003) consequent to administration of different herbal compounds has been reported before. *Aloe vera* inner gel possess some kinds of polysaccharides that have been shown to act as an immunostimulant and adjuvant in mammals, but the published data about *Aloe* effects on fish immune system are scarce (Kim *et al.*, 1999 and Alishahi *et al.*, 2010). Kim *et al.* used *Aloe vera* as a disease suppressing agent against *Vibrio alginolyticus* infection rather than as an immunoprophylactic (Kim *et al.*, 1999). In our previous work, we report some immunostimulatory effects of ACE in *C.carpio* but in this study the dose dependent response of *C.carpio* to ACE were evaluated (Alishahi *et al.*, 2010).

In the present study, it was observed that most of investigated immunological, haematological and growth parameters were affected in fish fed with 0.5 and 1% ACE supplemented food. According to the results, Food supplemented with 0.5% and 1% ACE, stimulates following growth

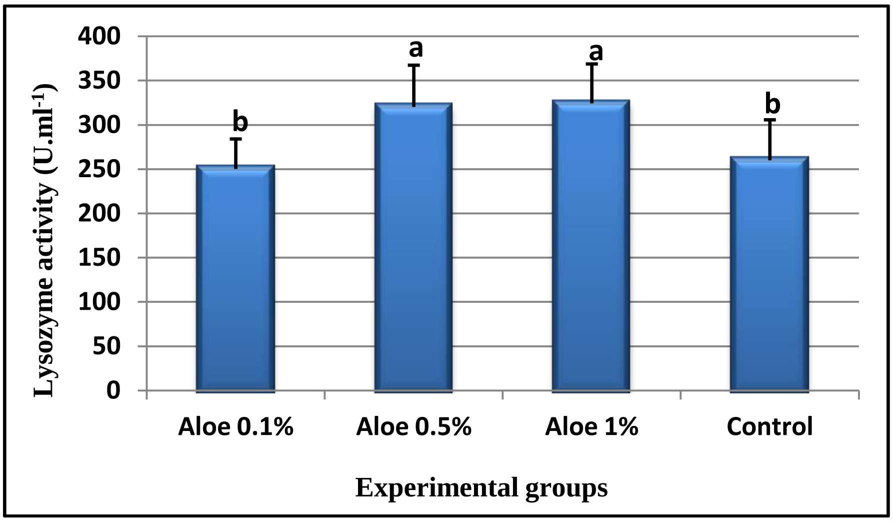


Figure 1. Results of lysozyme activity in 3 different concentrations of ACE and Control. Significant differences (p<0.05) are marked by different letters.

Table 1. detailes of growth indices, immune parameters and Hematological parameters are shown in this table. Significant differences (p<0.05) are marked by different letters.

Parameters	Control	ACE 0.1%	ACE 0.5%	ACE 1%
Growth indices				
Growth rate	82.16 ± 12.64 [b]	81/90 ± 14/01 [b]	102/5 ± 10.31[a]	91.89 ± 6.27 [a]
SGR	1.37 ± 0.211[b]	1.36 ± 0.233 [b]	1.71 ± 0.172 [a]	1.46 ± 0.105 [a]
FCR	3.1 ± 0.215 [b]	3.05 ± 0.201 [b]	2.44 ± 0.248 [a]	2.62 ± 0.091 [a]
Mortality	12 ± 3.33 [a]	10 ± 5.09 [a]	8.89 ± 5.09 [a]	10 ± 3.33 [a]
Immune parameters				
SBA assay	186.3 ± 22.7 [a]	191.4 ± 35.36 [a]	124.6±31.02 [b]	118.3 ± 23.9 [b]
NBT assay	0.312± 0.07 [b]	0.306± 0.05 [b]	0.395± 0.07 [a]	0.355± 0.06 [ab]
Complement activity	408 ± 18.2 [a]	402 ± 31.27 [a]	443.3± 30.03[b]	462.3 ± 31.2 [b]
Total protein (g dl^{-1})	3.26 ± 0.51 [a]	2.97 ± 0.38 [a]	3.59 ± 0.693 [b]	3.61 ± 0.642 [b]
IgM (g dl^{-1})	1.86 ± 0.55 [a]	1.68 ± 0.39 [a]	2.32 ± 0.559 [b]	2.77 ± 0.767 [b]
Hematological parameters				
PCV%	31.4 ± 8.48 [a]	32.7 ± 8.24 [a]	39.8 ± 6.49 [b]	43 ± 7.43 [b]
Hb	8.15 ± 1.36 [a]	8.06 ± 1.65 [a]	8.98 ± 1.91 [ab]	9.57 ± 1.75 [b]
MCV (fl)	246.7 ± 65.7 [a]	281.7 ± 92.6 [ab]	305.8 ± 42.56[b]	311 ± 80.57 [b]
MCH (pg)	64.9 ± 16.6 [a]	68.5 ± 18.58 [a]	68.8 ± 14.25 [a]	69.1 ± 18.3 [a]
MCHC (%)	27.6 ± 8.35 [a]	26.1 ± 6.13 [a]	22.9 ± 5.52 [a]	22.7 ± 5.46 [a]
RBC ($\times 10^6$ cell/mm^3)	1.29 ± 0.22 [a]	1.21 ± 0.21 [a]	1.31 ± 0.19 [a]	1.44 ± 0.33 [b]
Heterophil (%)	16 ± 2.54 [a]	18.8 ± 3.86 [a]	17.2 ± 2.98 [a]	17.5 ± 3.72 [a]
Lymphocyte (%)	83.2 ± 3.17 [a]	80 ± 3.94 [a]	81.1 ± 4.64 [a]	81.5 ± 3.96 [a]
Monocyte (%)	0.4 ± 0.51 [a]	0.8 ± 0.68 [a]	0.73 ± 0.7 [a]	0.73 ± 0.59 [a]
Eosinophil (%)	0.4 ± 0.51 [a]	0.33 ± 0.49 [a]	0.33 ± 0.49 [a]	0.33 ± 0.62 [a]

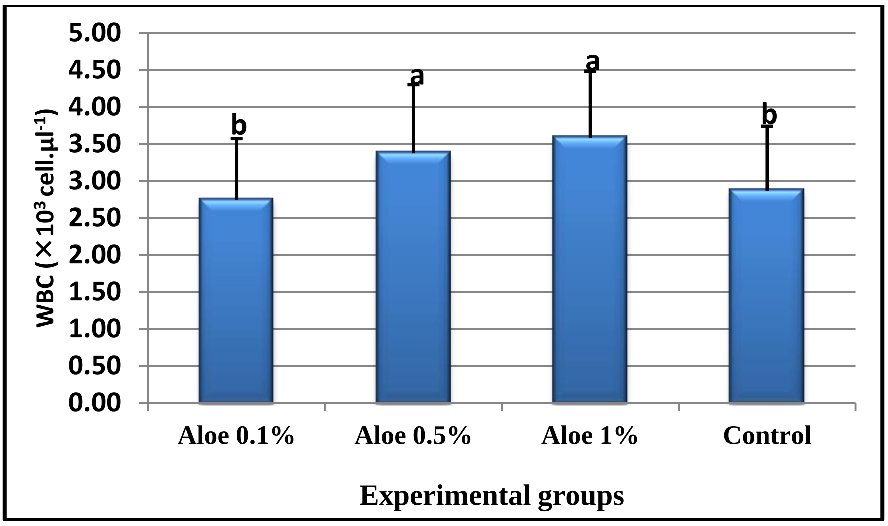


Figure 2. Data of WBC count is shown. As we can see, groups 0/5% and 1% significantly have higher levels of WBC (P<0.05).

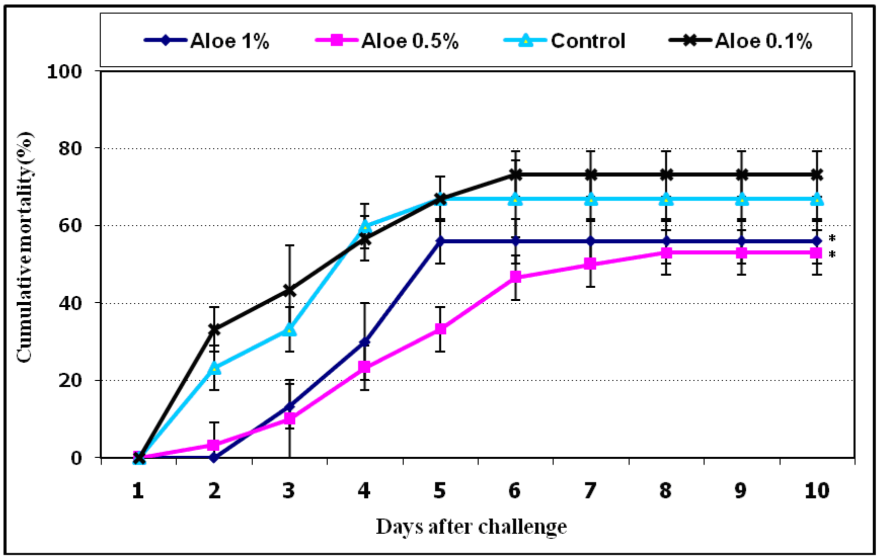


Figure 3. Mortality rate in group 0/5% and 1% ACE after challenge with bacteria is lower than two other groups and this difference is statistically significant (P<0.05). * shows significant difference with control group.

indices: AWG, SGR, FCR and FER in *C.carpio* fed for up to 60 days. Several authors have reported relationships between the use of immunostimulants and promotion in growth rate (Citarasu *et al.*, 2003).

According to the results of present study lysozyme activity enhanced in groups 3 and 4. Lysozyme is a very important part of immune system as it is widely distributed in serum, tissues and mucus. It is effective in lysis of not only Gram-positive bacteria but also Gram-negative microbes. lysozyme may become effective after complement and other enzymes have unmasked inner peptidoglycan layer (Alishahi and Buchmann, 2006). Similar results of elevated lysozyme activity were observed in *Labeo rohita* fed with 0.5% of *Achyranthes aspera* seed (Rao *et al.*, 2006) and microbial levan (Gupta *et al.*, 2008), in *C. carpio* with dietary *Astragalus radix* and *Ganoderma lucidum* (Yin *et al.*, 2009).

Although supplemented diet with 0.5% and

1% ACE were significantly increased the serum bactericidal activity against *A.Hydrophila* (p<0.05), diet supplemented with 0.1% had no stimulating effect on serum bactericidal activity compared to the control group. Divyagnaneswari *et al.* (Divyagnaneswari *et al.*, 2007) in tilapia and Misra *et al.* (Misra *et al.*, 2006) in Indian major carp (*Labeo rohita*) reported an increase in serum bactericidal activity after administration of some herbal extracts.

NBT activity, an indicator of respiratory burst activity in phagocytes in fish (Secombes, 1996), significantly increased in fish fed with 0.5% ACE enriched diet compare to control group, but other level of ACE did not induce any significant change in NBT activity of leukocytes. This stimulatory effect may be due to the proliferative responses of leucocytes as reported in rainbow trout macrophages following in vitro treatment with *Glycyrrhiza glabra* extract (Jang *et al.*, 1995) or due to the enhancement in the respiratory burst activity and expression of interleukins in Ergosan injected fish (Peddie *et al.*, 2002). The similar result of enhanced leucocytes NBT activity in rainbow trout by dietary 1% aqueous extract of ginger roots (Dügenci *et al.*, 2003) and Zeranol (Keles *et al.*, 2002) were also reported.

Complement, another component of the non-specific humoral immune response, was also studied in the present work. Fish treated with 0.5 and 1% ACE supplemented food showed significant increase in the alternative pathways when compared with control fish. Increase in complement activity could be partly related to the rise in the complements component and their activity in the serum. Thus, complement activity could contribute to protection against bacterial infection in ACE treated groups. Although in some similar works increase in complement activity were reported (Boshra *et al.*, 2006 and Cheng *et al.*, 2007), there are other studies found that oral administration of immunostimulants to turbot (Baulny *et al.*, 1996) and *C.carpio* (Selvaraj *et al.*, 2005 and Alishahi *et al.*, 2010) did not induce any change in complement activity.

Although total serum protein contents and total globulin were markedly increased in fish treated with 0.5 and 1% ACE supplemented food, change in serum protein and globulin content was independent to the dosages of ACE. The increase in serum protein content might be correlated with an increase of other proteins like serum lysozyme, complement factors and bactericidal peptides. Elevated total protein and globulin were reported following administration of medicinal plants in fish (Misra *et al.*, 2006; Alishahi *et al.*, 2010 & 2011).

In this study significant increase in some haematological parameters (PCV, RBC, WBC and Hb) was seen in groups 3 and 4. PCV and RBC are general indicators for fish health and help to describe abnormalities caused by immunostimulants (Selvaraj *et al.*, 2005). The main reason of this promotion can refer to the effect of Aloe on fish haematopoetic activity, and the increase in the leucocyte count might have resulted in the enhancement of the immune responce, because phagocytic cells are the key elements in the immune system and are the major affector and effector cells on which Aloe exerts its activities. Although WBC value showed significant increase in groups 3 and 4 (p<0.05), no significant change observed in types of leucocytes (lymphocytes, neutrophils, monocytes and eosinophiles) among the experimental groups (p>0.05).

For testing efficacy, it is very essential to estimate the increased protection in fish treated with immunostimulants (Sakai *et al.*, 2001). The present study revealed that oral administration of 0.5 and 1% ACE significantly improved the survival rate of *C.carpio* against challenge with the pathogen *A.hydrophila.* The enhancement of nonspecific immune parameters by 0.5 and 1% ACE incorporated in food is possibly an important factor in reducing the percentage mortality and thereby protecting the fish against live *A.hydrophila* challenge. The present finding is in agreement with the results of Abutbul *et al.* (Abutbul *et al.*, 2004) in tilapia fed with a diet containing extract of *Rosmarinus officinalis* and

Fujiki *et al.* (Fujiki *et al.*, 1994) in carp administered with the *Undaria pinnatifida*. In another study fish resistance against *A. hydrophila* was enhanced in *L.rohita* fed with 0.5% of Achyranthes (Rao *et al.*, 2006). Besides the methanolic herbal extracts of *Andrographis paniculata* and *Psoralea corylifolia* helped to increase the survival and growth and reduced the bacterial load even in the shrimp, *Penaeus monodon*, post larvae (Citarasu *et al.*, 2003).

To conclude, we have found that although oral administration of *Aloe vera* Crud Extract or ACE (0.5% and 1% in food) had stimulating effects on growth performance and hematoimmunological parameters of the *C.carpio*, food supplemented with 0.1% didn't show such effects. There was no significant difference in assayed parameters between fish fed with food supplemented with 0.5% and 1%, thus 0.5% ACE supplementation of food can be recommended as an immunostimulant in common carp.

Acknowledgments

This work was financially supported by the research council of Shahid Chamran University of Ahvaz, Ahvaz, Iran.

References

Abutbul, S., Golan-Goldhirsh, A., Barazani, O. and Zilberg D. (2004) Use of Rosmarinus officinalis as a treatment against Streptococcus iniae in tilapia (*Oreochromis* sp.). *Aquaculture* **238**, 97-105.

Alishahi, M. and Buchmann, K. (2006) Temperature-dependent protection against *Ichthyophthirius multifiliis* following immunization of rainbow trout using live theronts. *Diseases of Aquatic Organisms* **72**, 269-273.

Alishahi, M., Ranjbar, M.M., Ghorbanpour, M., Peyghan, R., Mesbah, M. and Razi jalali, M. (2010) Effects of dietary *Aloe vera* on specific and nonspecific immunity of Common carp (*Cyprinus carpio*). *Journal of Veterinary Researches* **4, 3**, 85-91.

Alishahi, M., Soltani, M. and Mesbah, M. (2011) Effects of Dietary *Silybum marinum* extract on immune parameters of the *Cyprinus carpio*. *Journal of Veterinary Research* **65, 3**, 255-263

Anderson, D.P. and Siwicki, A.K. (1994) Duration of protection against *Aeromonas salmonicida* in brook trout immunostimulated with glucan or chitosan by injection and immersion. *Progressive Fish Culturist* **56**, 258-261.

Azza, M.M. (2009) Antagonism of *Aeromonas hydrophila* by propolis and its effect on the performance of Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology* **27**, 454-459.

Baulny, M.D., Quentel C., Fournier V., Lamour F. and Gouvello R.L. (1996) Effect of long-term oral administration of b glucan as an immunostimulant or an adjuvant on some non-specific parameters of the immune response of turbot *Scophthalmus maximus*. *Disease of Aquatic Organism* **26**,139-147.

Boshra, H., Li, J. and Sunyer, J.O. (2006) Recent advances on the complement system of teleost fish. *Fish & Shellfish Immunology* **20**, 239-262.

Boudreau, M.D. and Beland, F.A. (2006) An evaluation of the biological and toxicological properties of *Aloe barbadensis* (miller). *Journal of Environmental Science and Health* **24**, 103-154.

Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein. *Annals Biochemistry* **72**, 248.

Chen, X., Wu, Z. and Yin, J. (2003) Effects of four species of herbs on immune function of *Carassius auratus gibelio*. *Journal of Fish Sciences of China* **10**, 36-40.

Cheng, A.C., Tu, C.W., Chen, Y.Y., Nan, F.H. and Chen, J.C. (2007) The immunostimulatory effects of sodium alginate and κ-carrageenan on orange-spotted grouper *Epinephelus coicoides* and its resistance against *Vibrio alginolyticus*. *Fish & Shellfish*

Immunology **22,** 197-205.

Citarasu, T., Venkatramalingam. K., Babu. M.M., Sekar, R.RJ. and Petermarian, M. (2003) Influence of the antibacterial herbs, *Solanum trilobatum*, *Andrographis paniculata* and *Psoralea corylifolia* on the survival, growth and bacterial load of *Penaeus monodon* post larvae. *Aquaculture International* **11**, 583-595.

Divyagnaneswari, M., Christybapita, D. and Michael, R.D. (2007) Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. *Fish & Shellfish Immunology* **23**, 249-259.

Dügenci, S.K., Arda, N. and Candan, A. (2003) Some medicinal plants as immunostimulant for fish. *Journal of Ethnopharmacology* **88**, 99-106.

Egger, S.F., Brown, G.S., Kelsey, L.S., Yates, K.M., Rosenberg, L.J. and Talmadge, J.E. (1996) Studies on optimal dose and administration schedule of a hematopoietic stimulatory beta-(1,4)-linked mannan. *International Journal of Immunopharmacology* **18(2)**, 113-126.

Ellis, A.E. (1990) Lysozyme assays. In: Stolen, J.S.; Fletcher, T.C.; Anderson, D.P.; Roberson, B.S.; van Muiswinkel, W.B. (eds.), Techniques in fish immunology. New Jersey. SOS Publications p. 101-113.

Fox, H.E., White, S.A., Koa, M.F. and Fernald, R.D. (1997) Stress and dominance in a social fish. *The Journal of Neuroscience* **16(17)**, 6463-6469.

Fujiki. K., Matsuyama. H. and Yano, T. (1994) Protective effect of sodium alginates against bacterial infection in common carp, *Cyprinus carpio*. *Journal of Fish Diseases* **17**, 349-355.

Goldenfarb, P.B., Bowyer, F.P. and Hall. T. (1971) Brosious, E.Reproducibility in the hematology laboratory: the microhematocrit determination. *American Journal of Clinical Pathology* **56**, 35–39.

Gupta, K.S., Pal, K.A., Sahu, P.N., Dalvi, R., Kumar, V. and Mukherjee, C.S. (2008) Microbial levan in the diet of *Labeo rohita* hamilton juveniles: effect on non-specific immunity and histopathological changes after challenge with *Aeromonas hydrophila*. *Journal of Fish Diseases* **31**, 649-657.

Hu, F., Hepburn, H.R., Li, Y., Chen, M., Radloff, S.E. and Daya, S. (2005) Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. *Journal of Ethnopharmacology* **100**, 276–283.

Jang, S.I., Marsden, M.J., Kim Y.G., Choi, M.S. and Secombes, C.J. (1995) The effect of glycyrrhizin on rainbow trout, *Oncorhynchus mykiss*, leucocyte responses. *Journal of Fish Disease* **18**, 307-315.

Jian. J. and Wu. Z. (2004): Influences of traditional Chinese medicine on non-specific immunity of Jian carp (*Cyprinus carpio* var. Jian). *Fish & Shellfish Immunology* **16**, 185-191.

Keles, O., Candan, A., Bakırel, T., and Karatas, S. (2002) The investigation of the anabolic efficiency and effect on the nonspecific immune system of zeranol in rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal of Veterinary and Animal Science* **26**, 925-931.

Kim, K.H., Hwang, Y.J., and C.Bai, S. (1999) Resistance to *Vibrio alginolyticus* in juvenile rockfish (*Sebastes schlegeli*) fed diets containing different doses of aloe. *Aquaculture* **180**, 13–21.

Krishnan, P. (2006) The scientific study of herbal wound healing therapies: Current state of play. *Current Anaesthesia & Critical Care* **17**, 21–27.

Lee, J.K., Lee, M.K., Yun, Y.P., Kim, Y., Kim, J.S. and Kim, Y.S. (2001) Acemannan purified from *aloe vera* induces phenotypic and functional maturation of immature dendritic cells. *International Immunopharmacology* **1(7)**, 1275–1284.

Misra. C.K., Das. B.K., Mukherjee. S.C. and Meher P.K. (2006) The

immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major carp, *Labeo rohita*. *Fish & Shellfish Immunology* **20**, 728-738.

Ortuno, J., Esteban, M.A., Mulero, V. and Meseguer, J. (1998) Methods for studying the haemolytic, chemoattractant and opsornic activities of seabream serum. In: Barnes, A.C., Davidson, G.A., Hiney, M.P., Mclntosh. D., editors. Methodology in fish diseases research, vol. I. Aberdeen Fisheries Research Services, 97-100.

Peddie, S., Zou, J. and Secombes, C.J. (2002) Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. *Veterinary Immunology and Immunopathology* **86**, 101-113.

Peng, S.Y., Norman, J., Curtin, G., Corrier, D., McDaniel, H.R. and Busbee, D. (1991) Decreased mortality of Norman Murine Sarcoma in mice treated with the immunomodulator, Acemannan. *Molecular Biotherapy* **3(2)**, 79.

Raa, J. (1996) The use of immuno-stimulatory substances in fish and shellfish farming. *Reviews in Fisheries Science* **4**, 229–288.

Raa, J., Roerstad, G., Engstad, R. and Robetsen, B. (1992) The use of immunostimulants to increase resistance of aquatic organisms to microbial infections Disease. *Asian Aquaculture* **1**, 39–50.

Rao, Y.V., Das, B.K., Jyotyrmayee, P. and Chakrabarti, R. (2006) Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish & Shellfish Immunology* **20**, 263-273.

Rojhan, M. (2007) Herbal drugs, 5th edition, farhikhtegan alavi publications, Tehran. Pg 287.

Sakai, M., Taniguchi, K., Mamoto, K., Ogawa, H. and Tabata, M. (2001) Immunostimulant effect of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio*. *Journal of Fish Diseases* **24**, 433-438.

Sattari, M. (2008) Ichthiology (2), 2nd edition, Haghshenas Publications, 2008, p. 191.

Schaperclaus, W., Kulow, H. and Schreckenbach, K. (1991) Hematological and serological technique. In: Kothekar VS, editor. Fish disease. 2nd ed. vol. 1. N, 56 Connaught circus, New Delhi: Gulab primlani, Oxonian press Pvt. Ltd; pp 71-108.

Secombes, C.J. (1996) The nonspecific immune system: cellular defenses. In: Iwama, G., Nakanishi, T., editors. The fish immune system. Organism, pathogen and environment. San Diego: Academic Press pp.63-105.

Selvaraj, V., Sampath. K. and Sekar. V. (2005) Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (*Cyprinus carpio*) infected with Aeromonas hydrophila. *Fish & Shellfish Immunology* **19**, 293-306.

Shelton, R.M. (1991) *Aloe vera*. Its chemical and therapeutic properties. *International Journal of Dermatology* **30**, 679-683.

Swain, P.S., Dash, P.K., Sahoo, P., Routray, S.K., Sahoo, S.D.,Gupta., Meher, P.K. and Sarangi, N. (2006) Non-specific immune parameters of brood Indian major carp Labeo rohita and their seasonal variations. *Fish & Shellfish Immunology* **22**, 38-43

Tan, B.K., and Vanitha, J. (2004) Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. *Current Medicinal Chemistry* **11(11)**, 1423-1430.

Thrall, M.A. (2004) Veterinary Hematology And Clinical Chemistry.Lippincott Whiliams & Wilkins ,USA, pp:241-288.

Yin, G., Ardo, L., Thompson, K.D., Adams, A., Jeney, Z., Jeney, G. (2009) Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. *Fish & Shellfish Immunology* **26**, 140-145.

Zapata, A., Diez, B., Cejalov, T., Gutierrez-defrias, C. and Cortes, A. (2006) Ontogeny of immune system of fish. *Fish & shell fish immunology* **20**, 126-36.

Iranian Journal of Veterinary Science and Technology
Vol. 5, No. 2, 2013, 33-44

IJVST

بررسی اثر غلظت‌های مختلف عصاره‌ی خام گیاه الوئه ورا به عنوان مکمل غذایی بر شاخص‌های رشد فاکتورهای ایمنی‌شناسی و خون‌شناسی ماهی کپور معمولی

مجتبی علیشاهی[1*]، اسماعیل عبدی[2]

[1] گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید چمران اهواز، اهواز، ایران
[2] دانشجوی دکتری تخصصی بهداشت و بیماریهای آبزیان، دانشکده دامپزشکی دانشگاه تهران ، تهران ، ایران

دریافت مقاله : ۱۳۹۱/۰۷/۲۳ پذیرش نهایی: ۱۳۹۲/۰۷/۱۰

چکیده

تحقیق حاضر با هدف بررسی تاثیر عصاره‌ی خام الوئه ورا بر اندیس‌های رشد، خون‌شناسی، ایمنی‌شناسی و میزان مقاومت در برابر عفونت باکتریایی *ائروموناس هیدروفیلا* در ماهی کپور معمولی صورت پذیرفت.۳۶۰ ماهی جوان بصورت تصادفی به ۴ گروه تقسیم شدند. گروه های یک تا چهار به ترتیب با مقادیر ۰، ۰/۱، ۰/۵ و ۱ درصد عصاره‌ی خام الوئه ورا در غذا به مدت ۶۰ روز تغذیه شدند. در پایان دوره شاخص‌های رشد شامل نرخ رشد ویژه، ضریب تبدیل غذایی، ضریب کارایی غذایی و درصد افزایش وزن محاسبه گردیدند. همچنین با اخذ نمونه‌های خون از ماهی‌ها پارامترهای خون‌شناسی شامل تعداد گلبول‌های قرمز و سفید خونی، شمارش افتراقی گلبول‌های سفید، هموگلوبین، هماتوکریت، متوسط حجم گلبولی، میزان هموگلوبین گلبولی و غلظت متوسط هموگلوبین گلبولی محاسبه شد. فاکتورهای ایمنی‌شناسی مورد بررسی نیز شامل میزان فعالیت لیزوزوم سرم، فعالیت باکتری‌کشی سرم، فعالیت الترناتیو کمپلمان، فعالیت انفجار تنفسی و میزان پروتئین و گلوبولین تام سرم بودند. نهایتاً ماهی‌ها با باکتری زنده‌ی ائروموناس هیدروفیلا مورد چالش قرار گرفته و تلفات تجمعی محاسبه گردید.

مطابق نتایج بدست امده اندیس‌های رشد بطور معنی‌داری در گروه ۳ و ۴ بهبود یافت ($p<0.05$) همچنین هماتوکریت و متوسط حجم گلبولی به همراه اغلب فاکتورهای ایمنی‌شناسی نیز در این گروه‌ها بهبود یافته بود ($p<0.05$). تلفات حاصل از چالش نیز در این گروه‌ها پائین تر از گروه کنترل بود ($p<0.05$). بنابراین می‌توان بیان نمود که افزودن عصاره‌ی الوئه ورا به میزان ۰/۵ و ۱ درصد به جیره‌ی غذایی اثرات بهبود رشد و تحریک ایمنی را دارا می‌باشد و باعث افزایش مقاومت ماهی در مقابل عفونت باکتریایی می‌گردد.

واژگان کلیدی: عصاره‌ی *الوئه ورا*، کپور معمولی، *ائروموناس هیدروفیلا*، محرک ایمنی، فاکتورهای هماتوایمونولوژی